Development of Selective Cathepsin Inhibitors for PET Imaging in Cancer Diagnosis

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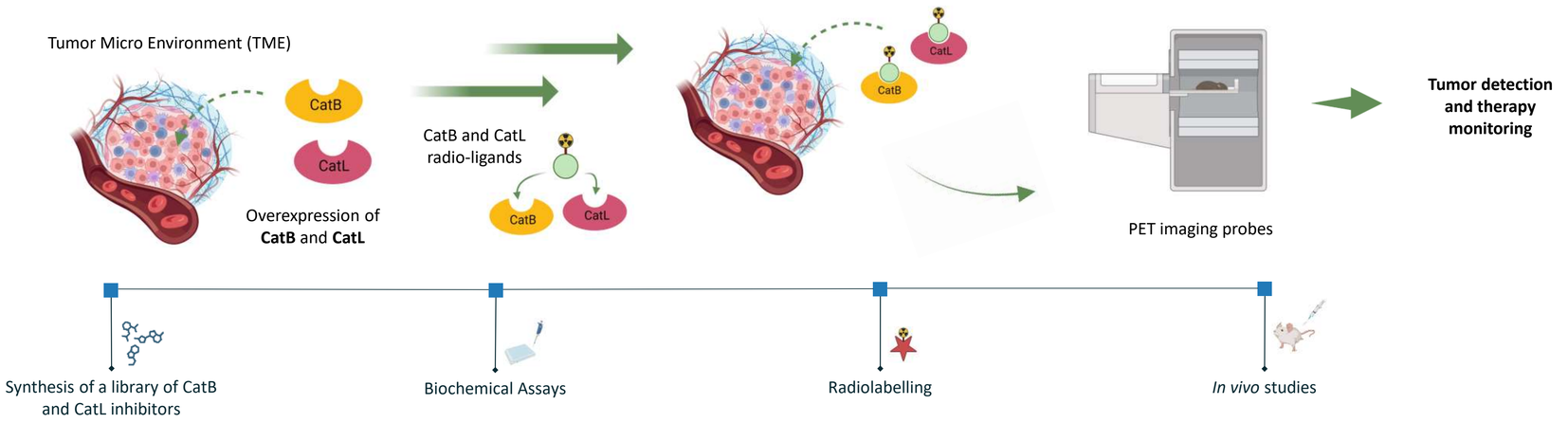
Introduction

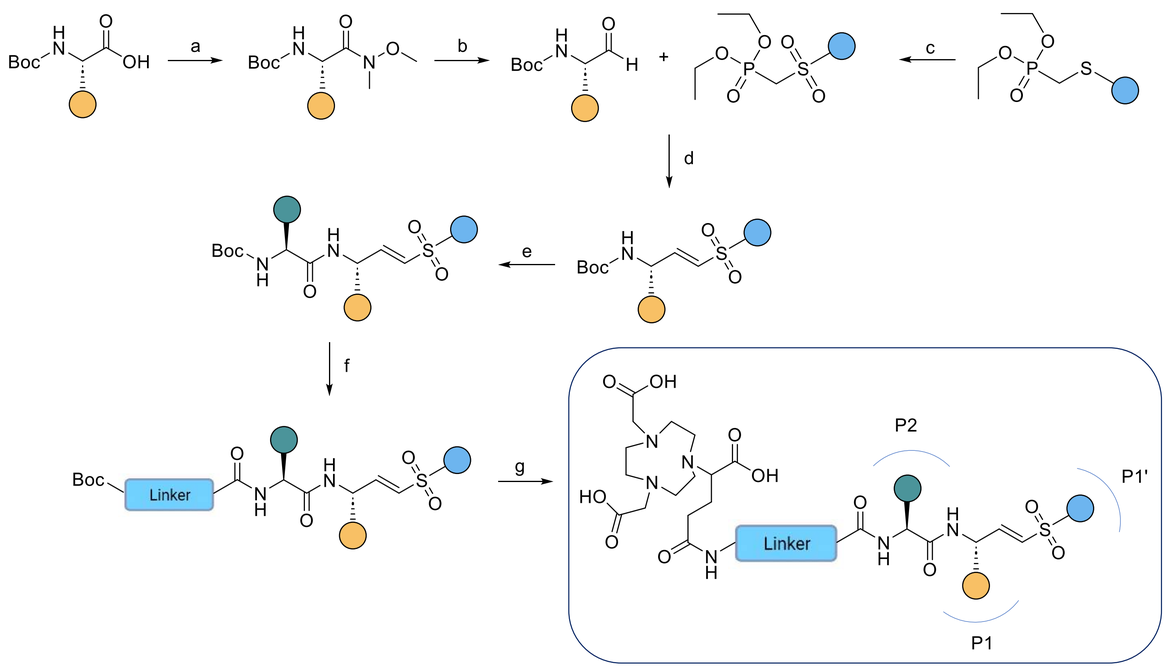
Cathepsins, a family of lysosomal cysteine proteases, are involved in critical biological processes, including protein turnover, extracellular matrix remodeling, and antigen processing.



In cancer, the **overexpression of cathepsins**, particularly Cathepsin B (CatB) and Cathepsin L (CatL), is associated with tumor progression, metastasis, and immune modulation. These enzymes contribute to tumorigenesis, invasion, and angiogenesis, making them highly relevant **biomarkers for cancer diagnosis**. The aim of this project is to develop **selective inhibitors of CatB and CatL**, which can be used to create **innovative imaging probes for Positron Emission Tomography (PET) imaging** and further enhance the management of cancer patients.

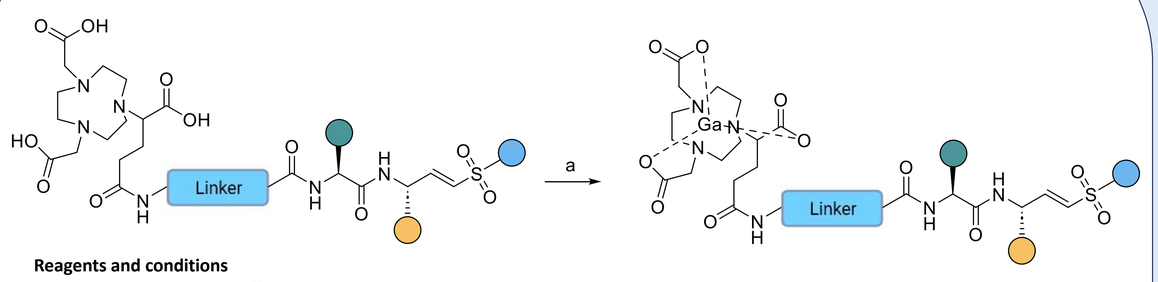
OncoProTools is an **MSCA Doctoral Network** that aims to develop tumor targeting strategies for **cancer diagnostics and therapeutics**, to make them more effective, selective, patientfriendly and personalized.

Synthesis of Cathepsin Inhibitors



Reagents and conditions
a. N,O-dimethylhydroxylamine hydrochloride, TEA, TBTU, HOBt, DCM, rt, ov., **b**. $LiAlH_4$, THF, 0°C, 1h. **c**. CH_3COOH, H_2O_2 30%, 85°C, 2h30min. **d**. NaH, THF, 0°C then rt, 3h. **e**. 1) TFA : DCM (1:1), rt, 2h. 2) Boc-AA, TEA, HOBt, TBTU, DMF, rt, ov. **f**. 1) TFA : DCM (1:1), rt, 2h. 2) Boc-Linker, DIPEA, TBTU, DMF, rt, ov. / Boc-Linker, DIPEA, THF, -15°C then rt, ov. **g**. 1) TFA : DCM (1:1), rt, 2h. 2) NODA-GA NHS ester, DIPEA, DMF, rt, ov.

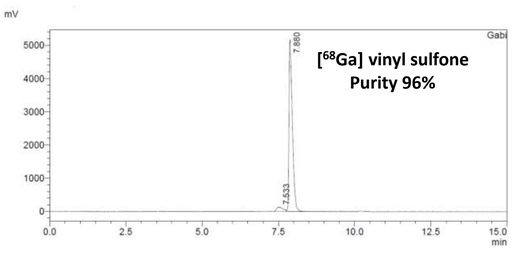
^{68}Gallium radiolabeling



Reagents and conditions
a. $^{68}GaCl_3$, 80°C, NH_4OAc buffer, pH 5.5, 5 min.

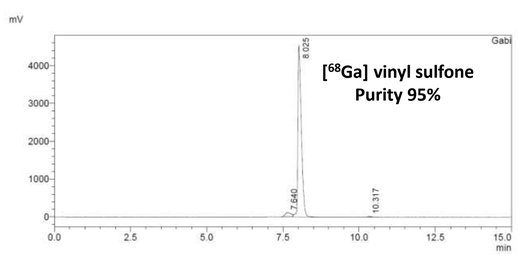
[^{68}Ga]CREANT-101

Radiochemical conversion	Purification	Yield
94%	tC2 cartridge	87%

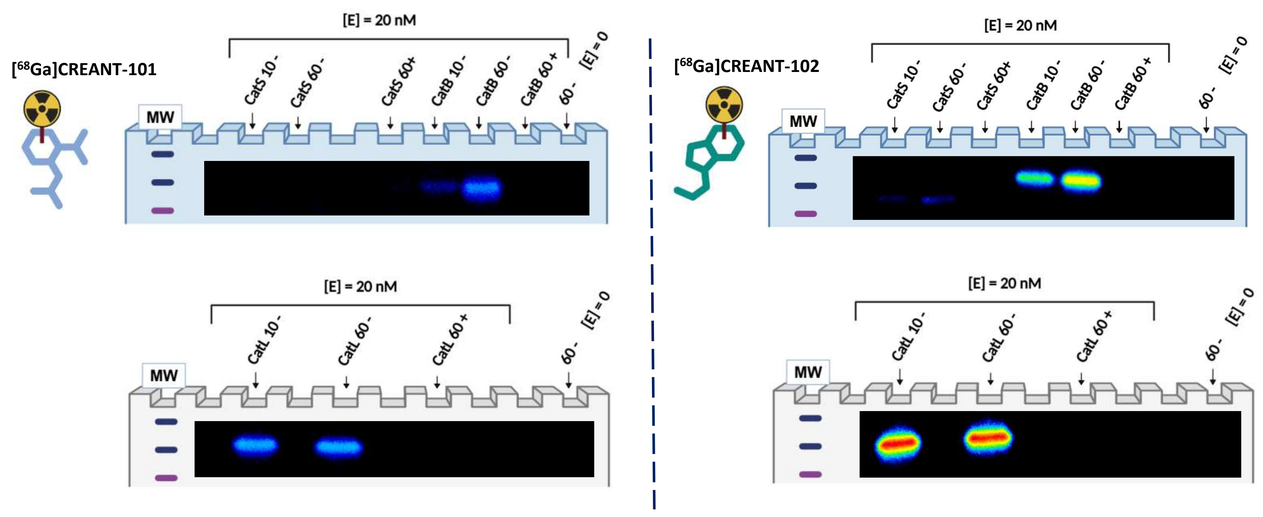


[^{68}Ga]CREANT-102

Radiochemical conversion	Purification	Yield
90%	tC2 cartridge	78%

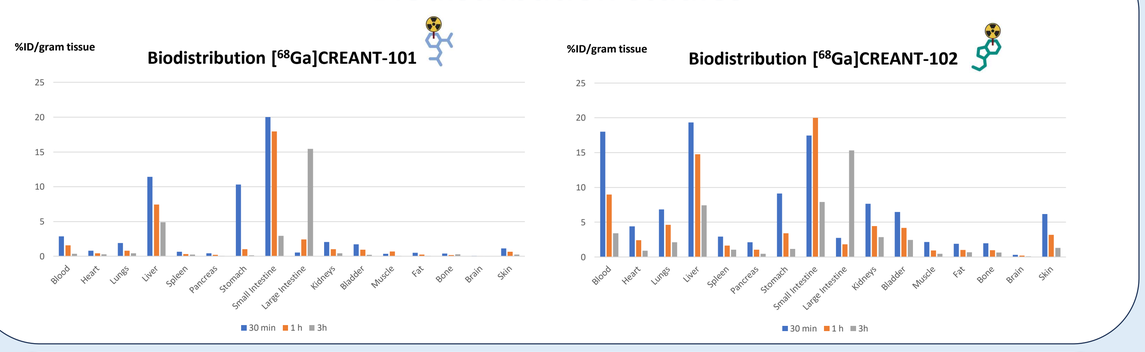


SDS-PAGE autoradiography

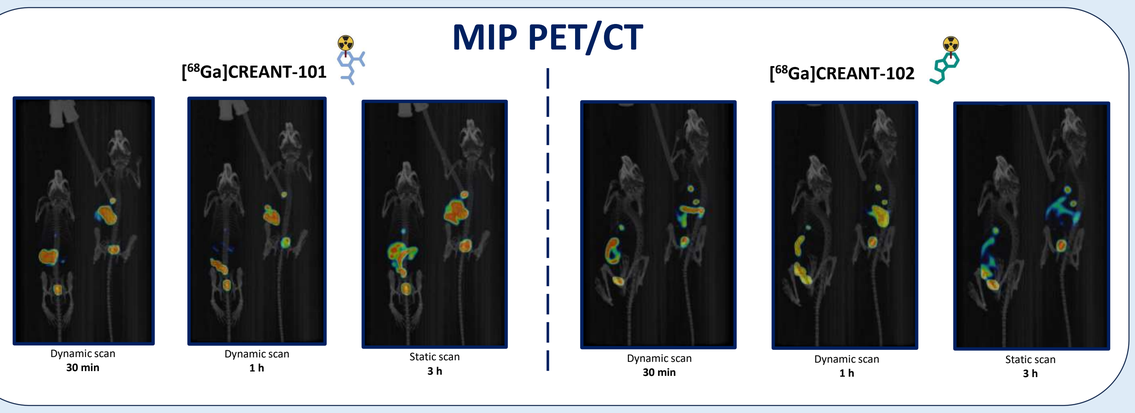


Reference: **(+)** preincubated with K11777 for 30 minutes at 37 °C; **(-)** no preincubation; **10** - incubated for 10 minutes at 37 °C; **60** - incubated for 60 minutes at 37 °C.

Biodistribution studies



MIP PET/CT



Conclusion and Outcomes

The developed vinyl sulfone-based probes demonstrated **high selectivity for CatL**, with minimal off-target binding, as confirmed by enzymatic assays and SDS-PAGE. *In vivo* studies revealed **distinct pharmacokinetics and biodistribution patterns**, offering valuable insights for further optimization. Future PET imaging studies in **tumor models** will focus on refining their selectivity and improving their diagnostic performance for enhanced cancer imaging.

References
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- *Schleyer, K. A.; Cui, L. Molecular Probes for Selective Detection of Cysteine Cathepsins. Org. Biomol. Chem. 2021, 19 (28), 6182–6205.*

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